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APPLICATION NUMBER: *60/463,620*
FILING DATE: *April 16, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/11929*

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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 976463865 US

U.S. PTO J1011 04/16/03 60463620

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TITLE OF THE INVENTION (500 characters max)

STABLE PEPTIDOMIMETIC OF THE RGD PEPTIDE SEQUENCE FOR CELL-MATRIX INTERACTION

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ENCLOSED APPLICATION PARTS (check all that apply)

☑ Specification *Number of Pages* 18 ☐ CD(s), Number

☐ Drawing(s) *Number of Sheets* ☐ Other (specify)

☐ Application Data Sheet. See 37 CFR 1.76

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☑ Applicant claims small entity status. See 37 CFR 1.27.

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FILING FEE AMOUNT ($): $80.00

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Respectfully submitted,

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Date 04/16/2003

REGISTRATION NO. (*if appropriate*) 30,346

Docket Number: ASU 3061

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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Docket No. ASU3061PRV
Inventor: Massia et al.

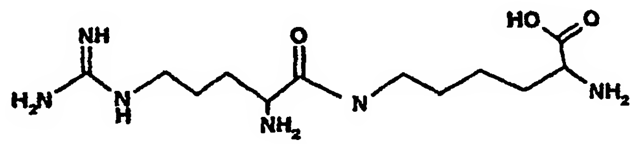
STABLE PEPTIDOMIMETIC OF THE RGD PEPTIDE SEQUENCE FOR CELL-MATRIX INTERACTION

FIELD OF INVENTION

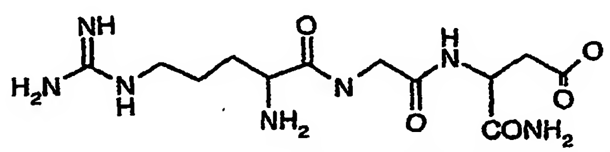
This invention concerns integrin-mediated cell adhesion processes implicated in cell migration, invasion, proliferation, angiogenesis, bone resorption, apoptosis and gene expression. Means and methods are provided for treatment of diseases caused by abnormal cell adhesion processes. Such diseases include, for example, tumor growth, cancer metastasis, thrombosis, occlusive cardiovascular disease, osteoporosis, retinopathy, renal failure and wound healing. An RGD peptide mimetic is provided that exhibits proteolytic stability under physiological conditions and thus is therapeutically useful in humans and animals. The immobilized RGD peptide mimetic is useful as a cell attachment substrate for cell culture materials and biomaterial implant applications.

Brief Summary of the Invention

The present invention relates to a novel peptidomimetic compound N-6-(5 guanidino-heptyl)-2, 6 diamino-hexanoic acid having the same integrin-binding activities as Arg-Gly-Asp (RGD)-containing synthetic peptides, but are very stable to proteolytic degradation in comparison to proteolytically unstable peptides. Thus, this novel non-peptide mimetic would have the broad therapeutic utility of the RGD peptides and have higher stability and therapeutic efficacy. This RGD peptide mimetic also is very simple to synthesize in comparison to the state-of-the art in the design and synthesis of RGD peptide mimetics. The structural formula of this compound (RGD mimetic) is as follows with the native RGD peptide sequence (RGD) as a reference:



(RGD mimetic)



(RGD)

Possible commercial applications of this invention include biopharmaceuticals for treatment of a vast number of diseases/pathologies including tumor growth, cancer metastasis, thrombosis, occlusive cardiovascular disease, osteoporosis, retinopathy, renal failure, and wound healing. This invention could also be utilized as cell attachment substrates for cell culture and biomaterial implant applications.

Detailed description of the invention

Background Information- Cell adhesion to both natural and synthetic substrates is mediated by cell adhesion proteins which are secreted by cells in tissues and are present in the extracellular matrix *in vivo* (Grinnell, 1978; Hay, 1981). Cell adhesion proteins are immobilized in the matrix by virtue of a high affinity interaction with major structural components of the extracellular matrix (ECM), such as collagens and proteoglycans. Examples of this family of adhesive proteins include fibronectin (FN), vitronectin (VN), collagens, thrombospondin, von Willebrand factor (vWF), elastin, and laminin (LN). Cell adhesion to synthetic surfaces is also mediated by cell adhesion proteins (Grinnell, 1976a,b; Horbett & Schway, 1988). In this case, cell adhesion proteins which are dissolved in the fluid (such as culture medium or body fluids) adsorb to the material surface and support cell adhesion and spreading. Several classes of cell-surface receptors have a high affinity for these cell adhesion proteins, and it is by this affinity that cells adhere and spread on the ECM and synthetic substrates (Pearlstein, 1976; Kleinman *et al.*, 1976; Juliano, 1987). Some cell adhesion receptors recognize and bind to a select group of adhesion proteins, whereas other more promiscuous receptors bind to a wide variety of adhesion proteins. Fibronectin was the first cell adhesion protein that was shown to be involved in the

adhesion of cells to extracellular substrates (Pearlstein, 1976; Kleinman *et al.* 1976). *In vitro*, fibronectin is available from the serum in a form historically known as cold-insoluble globulin and now referred to as plasma fibronectin. For normal attachment and spreading to occur, plasma fibronectin must be adsorbed to the culture surface (Grinnell, 1976 a,b).

In order to determine the molecular basis of cell adhesion and related cellular activities, several laboratories employed the technique of limited proteolysis to isolate cell-adhesive domains in fibronectin (Hahn & Yamada, 1979; Ruoslahti & Hayman, 1979; McDonald & Kelley, 1980; Sekiguchi & Hakomori, 1980; Ehrismann *et al.*, 1981; Hayashi & Yamada, 1983). Assignment of cell-binding activity was based on assays measuring fibroblast attachment or spreading on fragment-coated substrates. Exhaustive peptic cleavage of a 120 kD fibronectin cell-binding fragment and evaluation of the cleaved subfragments revealed a 108 amino acid 11.5 kD fragment that supported cell adhesion (Pierschbacher *et al.*, 1982). From analyses of four synthetic peptides that together spanned the entire 11.5 kD fragment, one active site was localized to a 3.4 kD polypeptide at the C-terminus of the 11.5 kD fragment (Akiyama *et al.*, 1985a). Systematic testing of progressively smaller synthetic peptides, which were based on the 3.4 kD polypeptide sequence, subsequently identified a short determinant that now appears to represent the minimal active sequence within this region of fibronectin. The active site contains the tetrapeptide Arg-Gly-Asp-Ser (RGDS) (Pierschbacher & Ruoslahti, 1984a; Yamada & Kennedy, 1984). The biological interaction of the RGDS sequence with cell-surface fibronectin receptors was revealed by demonstrating that synthetic RGDS-containing peptides in solution could competitively inhibit fibroblast cell spreading on fibronectin-coated substrates (Pierschbacher & Ruoslahti, 1984a,b; Yamada & Kennedy 1984, 1985; Akiyama & Yamada 1985b; Hayman *et al.*, 1985a; Horwitz *et al.*, 1985; Silnutzer & Barnes, 1985). Soluble RGDS also inhibited the direct binding of radiolabeled fibronectin to fibroblastic cells in suspension (Akiyama & Yamada 1985c). These competition studies indicated that the RGD sequence is critical for the cell adhesive function of the parent molecule.

After the RGD cell adhesion recognition site in fibronectin was identified, the sequences of other cell adhesion proteins were examined for related signals. Other proteins known to carry functional RGD sequences include the platelet adhesion proteins fibrinogen and von Willebrand factor (Gartner & Bennett, 1985; Ginsberg *et al.*, 1985; Haverstick *et al.*, 1985; Plow *et al.*, 1985; Ruggeri *et al.*, 1986), type I collagen (Dedhar *et al.*, 1987), vitronectin (Hayman *et al.*, 1985b; Suzuki *et al.*, 1984), osteopontin (Oldberg *et al.*, 1986), and laminin (Grant *et al.*, 1989). These findings imply that RGD is a ubiquitous cell adhesion signal.

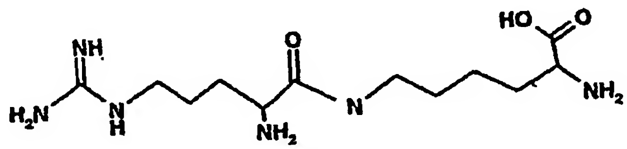
Isolation of RGD-directed cell-surface receptors for various cell adhesion proteins from many cell types was performed using affinity chromatography on Sepharose carrying the appropriate, covalently bound, adhesion protein. Cell-surface adhesion receptors from cell extracts were observed to specifically bind to these columns and were eluted with RGD-containing peptide solutions. The use of fibronectin as the affinity ligand yielded a receptor that was a heterodimer with a 160 kD α-subunit and a 140 kD β-subunit (Pytela *et al.*, 1985a, 1986). Similar affinity chromatography experiments have yielded distinct heterodimeric RGD-directed receptors specific for vitronectin (Pytela *et al.*, 1985b) and a platelet receptor with affinities for fibrinogen and fibronectin (Gardner & Hynes, 1985; Pytela *et al.*, 1986). It was realized that the heterodimeric structure was characteristic of RGD-directed receptors, with α-subunits ranging between 140 and 160 kD and β-subunits ranging between 90 and 140 kD. These RGD receptors, known as integrins, form the integrin superfamily of cell-surface adhesion proteins (Hynes, 1987; Ruoslahti & Pierschbacher, 1987).

The integrin superfamily is an important and well characterized group of cell-surface receptors for both cell-substrate and cell-cell adhesion (reviewed in: Hynes, 1987; Ruoslahti & Pierschbacher, 1987; Albelda & Buck, 1990; Humphries, 1990; Akiyama *et al.*, 1990; Ruoslahti, 1991; Tucker, 2002). Integrins are characteristically membrane-spanning heterodimeric protein complexes consisting of a α subunit and a β subunit. 18 distinct α subunits and 8 distinct β subunits have currently been isolated and identified, and some 24 $\alpha\beta$ combinations have been observed. Integrin complexes containing $\beta1$ and $\beta3$ subunits generally are involved in cell adhesion to the extracellular matrix, while the $\beta2$ integrins are involved in cell-cell adhesion. The complement of integrins expressed by different cell types varies greatly. Mammalian cells can express from two to ten different integrins, depending on the cell type (Humphries, 1990), and provide a means by which the cell senses its local environment and responds to changes in extracellular matrix composition and topography. Integrins were initially identified as cell-surface adhesion receptors mechanically linking the cytoskeleton to the extracellular matrix or to other cells. Now integrins are also recognized as cell signaling receptors implicated in the regulation of cellular adhesion, migration, invasion, proliferation, angiogenesis, bone resorption, apoptosis, and gene expression (Tucker, 2002).

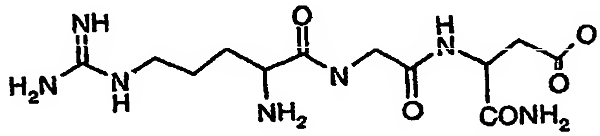
With the discovery of integrins and RGD as the major receptor-ligand system for promoting cell-ECM interactions and some cell-cell interactions, it was soon realized that many cellular events were regulated by integrin-mediated cell attachment to ECM, e.g. cell migration, growth, differentiation, and apoptosis. Thus, reagents containing or mimicking the RGD sequence could be developed to promote cell adhesion when such reagents are immobilized on solid surfaces and inhibit cell adhesion when in a soluble form (Ruoslahti, 1996). Therapeutic applications for soluble RGD-containing and RGD –mimetic reagents include tumor growth, cancer metastasis, thrombosis, occlusive cardiovascular disease, osteoporosis, retinopathy, renal failure, inflammation, infection, and wound healing (Craig, 1995). RGD peptide surface coatings on biomedical implant materials have been shown to promote faster and more complete tissue integration at the implant/tissue interface and reduce the foreign body response (Craig, 1995).

Recent advances in cell/ECM adhesion research have provided important insights into the role of integrin/matrix interactions in disease processes and have lead to the development of biotherapeutics (Lobb, 2002). Candidate reagents for biotherapeutic applications include monoclonal antibodies that block integrin function and RGD peptides or peptidomimetics (Engleman, 1996). Peptidomimetics are of particular interest because they have low immunogenicity and are resistant to protease/peptidase-mediated degradation (Engleman, 1996). The major disadvantage to peptidomimetics is that are difficult to synthesize. The present invention is a novel RGD peptidomimetic that has a simple and efficient synthesis protocol.

Summary of the invention- The present invention relates to a novel peptidomimetic compound N-6-(5 guanidino-heptyl)-2, 6 diamino-hexanoic acid having the same integrin-binding activities as Arg-Gly-Asp (RGD)-containing synthetic peptides, but are very stable to proteolytic degradation in comparison to proteolytically unstable peptides. Thus, this novel non-peptide mimetic would have the broad therapeutic utility of the RGD peptides and have higher stability and therapeutic efficacy. This RGD peptide mimetic also is very simple to synthesize in comparison to the state-of-the art in the design and synthesis of RGD peptide mimetics. The structural formula of this compound (RGD mimetic) is as follows with the native RGD peptide sequence (RGD) as a reference:



(RGD mimetic)



(RGD)

Possible commercial applications of this invention include biopharmaceuticals for treatment of a vast number of diseases/pathologies including tumor growth, cancer metastasis, thrombosis, occlusive cardiovascular disease, osteoporosis, retinopathy, renal failure, and wound healing. This invention could also be utilized as cell attachment substrates for cell culture and biomaterial implant applications.

Detailed description of the preferred embodiments

Synthesis of N-6-(5 guanidino-heptyl)-2, 6 diamino-hexanoic acid- Solid phase synthesis was utilized to prepare this RGD peptidomimetic. Figure 1 schematically depicts the synthesis scheme where resin-bound lysine is covalently linked to arginine by forming an amide bond with the ε amine group on lysine with the α carboxy group from arginine. 5gr (0.5mmol/g) Boc-Lys (Fmoc)-Wang Resin (from Advanced ChemTech, Cat#SK5151, Lot#15727-1) was soaked in 20% piperidine in DMF (Advanced ChemTech, Cat# RC8206, Lot# 13815) for 30 minutes to swell and also to remove Fmoc group. The resin was then washed with DMF several times to remove all piperidine (at least 3 times). 2 equivalent (3.244 g) of Fmoc-Arg (Pbf)-OH (from Advanced ChemTech, Cat#FR2136) together with 2 equivalent of HATU (1.901 g) was prepared and was dissolved in 0.5 M Diisopropylethylamine (DIPEA) in DMF. (1 equivalent=2.5 mmole), 2 equivalent= 5 m mole) the total volume of the solvent was 21.8 ml). This solution was added to the washed resin and reacted for 45 min to 1 hour. The resin was then washed with DMF to remove residual unreacted Arg and HATU. 20% piperidine in DMF was added to remove Fmoc from resin-bound Arg alpha amino groups. The resin was then allowed to stand for 30 min followed by these washes: a) DMF to remove piperidine, and b) CH_2Cl_2 (dichloromethane) to remove DMF. The resins were then dried overnight under vacuum. To cleave the product from resin, TFA (Advanced ChemTech, Cat# RA8402, Lot#16978) containing triisopropylsilane and ethanedithiol (95:2.5:2.5) was added and left for 4 hours. Cleaved product, N-6-(5 guanidino-heptyl)-2, 6 diamino-hexanoic acid, was precipitated with ether and dried under vacuum overnight.

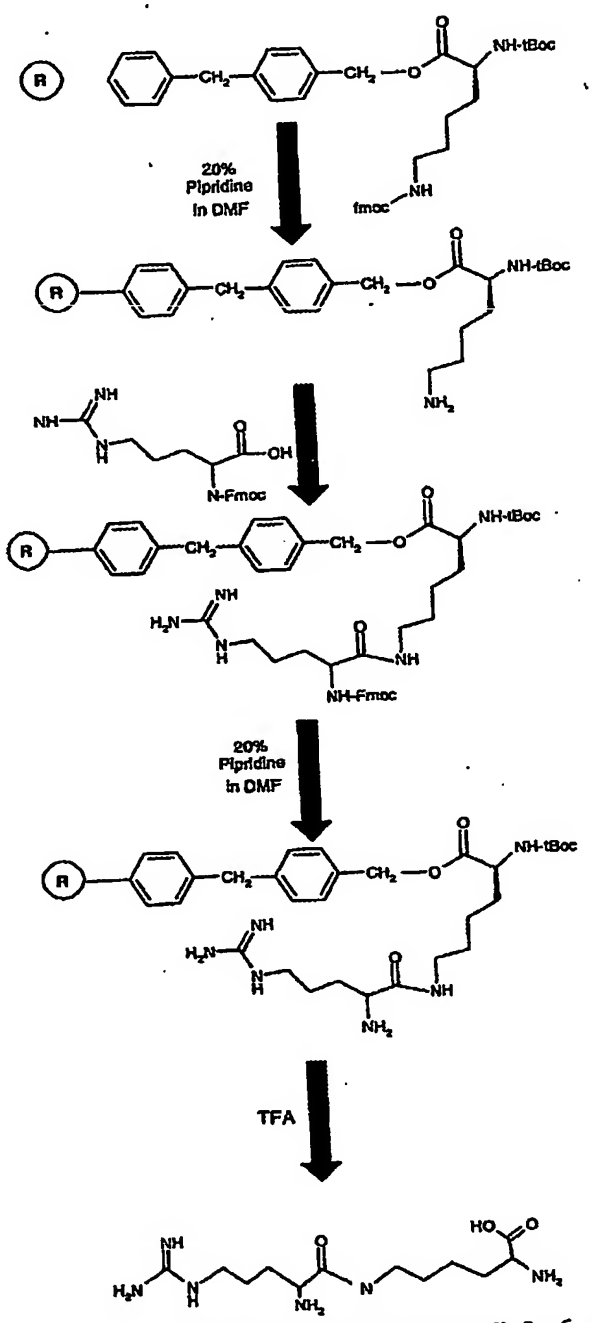


Figure 1. Solid phase synthesis of N-6-(5 guanidino-heptyl)-2, 6 diamino-hexanoic acid. (R represents Wang resin) Essentially resin-bound lysine is covalently linked to arginine by forming an amide bond with the ε amine group on lysine with the α carboxy group from arginine. The arginine-lysine is eventually cleaved from the resin as the final product, N-6-(5 guanidino-heptyl)-2, 6 diamino-hexanoic acid.

Experimental design- In vitro studies were undertaken to assess both the cell-adhesive properties of surface immobilized N-6-(5 guanidino-heptyl)-2, 6 diamino-hexanoic acid (GHDHA) peptidomimetic and the counteradhesive integrin antagonist activity of soluble GHDHA. GHDHA was covalently immobilized on dextran-coated tissue-culture plastic utilizing methods that we have developed for cell adhesion peptides. Cell adhesion was on surface-immobilized GHDHA and compared to surface-immobilized peptide GRGDSP. Other experiments were designed to assess the effect of soluble GHDHA on cell adhesion to GRGDSP to determine whether soluble GHDHA competitively inhibited intergin-RGD mediated cell adhesion or not. A similar experimental series was setup to determine whether soluble, integrin-binding GRGDSP competitively inhibited cell adhesion to surface immobilized GHDHA. Experimental methods, results, and discussion are presented in this section.

Method 1: Surface immobilization of GHDHA and GRGDSP

We recently reported a reductive amination method for immobilizing dextran to aminated glass surfaces and observed reduced cell adhesion on dextran-coated substrates (Massia, 2000). Greisser *et al.* has recently reported a similar method for immobilizing dextran to gas plasma-activated polymer surfaces (Greisser, 2000). For these studies, we immobilized non-peptide mimetic GHDHA and peptide GRGDSP on dextran-coated tissue culture plastic dishes. Multi-well cell culture dishes were first surface-aminated by adsorbing poly-lysine. Dextran was then immobilized on these surfaces using reported methods (Massia, 2000). GHDHA and GRGDSP were then surface-immobilized on dextran-coated tissue culture plastic using reported methods (Massia, 2001). Dextran-coated substrates were activated by oxidation of the glucose subunits (Glc) with sodium metaperiodate to convert Glc subunits to cyclic hemiacetal structures. Hemiacetal-containing subunits were then reacted with N-terminal amines of peptides forming an amine linkage between peptides and surface-immobilized dextran.

Periodate oxidation of dextran- Dextran was oxidized to produce aldehyde groups via standard periodate methods (Wilson, 1976). Dextran (M.W. 40 kDa, Sigma), 1g, was first dissolved in 30 ml deionized water. Sodium periodate ($NaIO_4$, 0.1M) was prepared for immediate use. This $NaIO_4$ solution was then added to the solution of dextran to make a 50% molar ratio of $NaIO_4$ to dextran (moles of glucose monomer). The reaction mixture was stirred at 4°C overnight and protected from light by covering the reaction flask with aluminum foil. The solution was then purified by precipitation of un-reacted periodate and iodate products using an equi-molar aqueous solution of $BaCl_2$. The purified oxidized dextran solution was then lyophilized and stored (if not immediately used) at 4°C in a 50mL conical centrifuge tube protected from light. The product was analyzed by FT-IR. The results showed a peak at 1700 nm indicating the aldehyde groups within the dextran chain.

Covalent Coupling of Oxidized Dextran to Surface-Aminated Cell Culture Dishes- Multi-well cell culture dishes were first surface-aminated by immediate immersion in 0.01% aqueous Poly-L-Lysine (PLL) solution then was incubated overnight (Fig. 2). Oxidized dextran, prepared as described above, was dissolved in 0.2 M sodium phosphate buffer (pH 9, 0.02 g/ml). Immediately following surface amination procedures, oxidized dextran solution (2 ml) was added to six well multiwell dishes containing surface-aminated substrates (Fig. 3). The substrates were allowed to incubate at room temperature for 16h on a rocker platform and protected from

light. Following incubation, the reaction mixture was decanted from the culture wells, and replaced by fresh 0.1M solution of sodium borohydride, $NaBH_4$ to reduce Schiff bases formed and to quench any free unreacted aldehyde groups present on the oxidized dextran chain. The substrates were allowed to incubate for 2 hours on the rocker platform. The $NaBH_4$ solution was then decanted and the substrates were rinsed gently several times with deionized water to remove unbound dextran (Fig. 3).

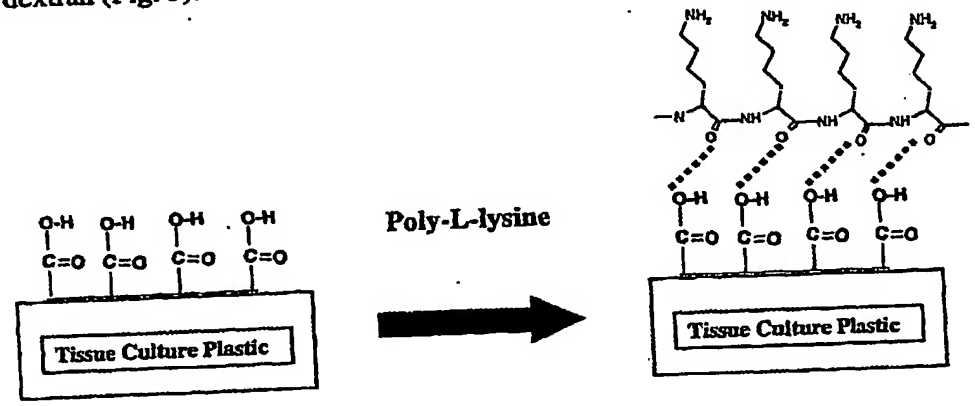


Figure 2. Introduction of surface amines on tissue culture plastic wells by the adsorption of polylysine.

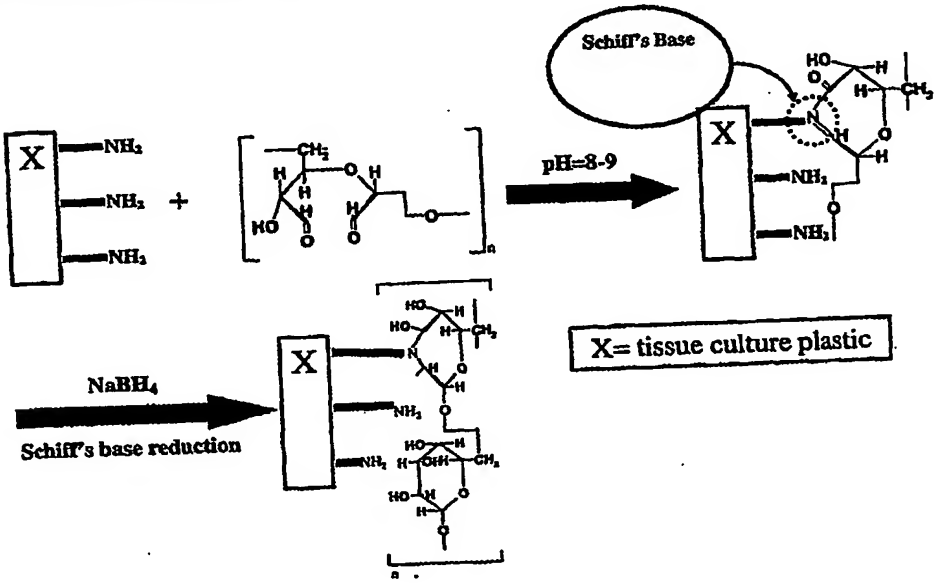


Figure 3. Reaction scheme for the surface immobilization of dextran to amine-bearing tissue culture plastic. Dextran is activated by oxidation of the glucose subunits (Glc) with sodium metaperiodate to convert Glc subunits to cyclic hemiacetal structures. Hemiacetal-laden dextran is then reacted with surface-bound amines forming Schiff base linkages. These linkages were immediately reduced via sodium borohydride forming more stabile amide linkages between dextran and surface-bound amines.

Covalent Coupling of GRGDSP and GHDHA- Surface grafting of peptide GRGDSP and peptidomimetic GHDHA to dextran-coated substrates was achieved via previous reported methods (Massia, 2001). Dextran-coated substrates committed for peptide/peptidomimetic grafting were oxidized with 0.1M sodium periodate, for one hour at room temperature, to activate substrate surfaces for covalent immobilization of peptides (Fig. 4). Following surface oxidation, samples were rinsed with deionized water and then peptide or peptidomimetic stock solutions (0.1mg/ml in 0.2M dibasic sodium phosphate, pH 9.0) were added to each sample well. A rocker table agitated the plates for 24h while being protected from light with a tin foil covering. Culture well substrates were decanted and rinsed with deionized water at the end of the 24h duration. Following peptide/peptidomimetic coupling, the substrates were incubated in dibasic sodium phosphate (0.2M, pH 9.0) containing 0.1M sodium borohydride, $NaBH_4$, to reduce Schiff bases formed and to quench any free unreacted aldehyde groups present. The substrates were allowed to incubate for 2-3 hours on the rocker platform. The substrates were rinsed with PBS and immediately were employed for in vitro experiments (Fig. 4).

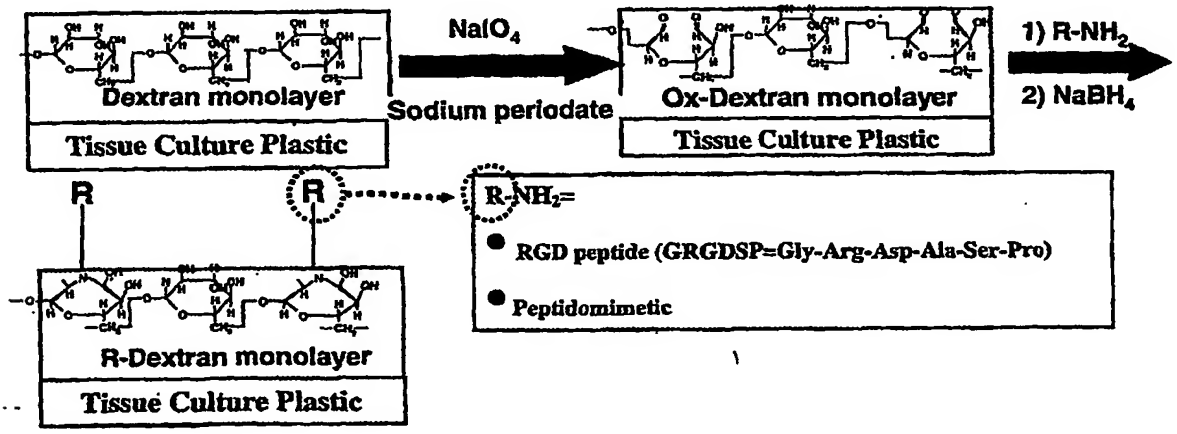


Figure 4. Reaction scheme for the surface immobilization of peptides to dextran-coated tissue culture plastic surfaces. Dextran-coated materials were reactivated by periodate oxidation and peptides were covalently linked by reacting N-terminal amines with hemiacetal structures in oxidized surface-bound dextran.

Method 2: Cell adhesion assays

Cell Culture- The cell line utilized in these studies was 3T3 fibroblasts (ATCC #CRL-6476. 3T3 cells were maintained in DMEM with 10% FBS at 37°C in saturated humidity. All cell culture media and reagents were obtained from Life Technologies, Inc.

Cell Adhesion Assay- Cell area coverage assays were performed to assess the extent of 3T3 cell adhesion and spreading on all substrates. 3T3 cells were seeded on 24-well culture dishes (15,000 cells/well) and incubated for 24 hrs. Following incubation, samples were fixed (3.8% formaldehyde in PBS, 5 min) and (0.1% aqueous toluidine blue, 5 min). Stained cells were then examined using phase contrast or stereomicroscopy (Leica) at 100x magnification. Three

random 100x fields were selected for each substrate for analysis. The extent of cell adhesion was determined for each captured digital image by calculating a percentage of cell area coverage using digital image analysis software. Final data was presented as a percentage of control adhesion. The percentage of control adhesion was calculated by multiplying the ratio of % area coverage on dextran-coated, peptide-grafted, or peptidomimetic-grafted substrates to % cell area coverage on untreated tissue culture plastic by 100. The average percentage of control adhesion was determined from duplicate independent experiments. Comparisons between samples groups were made using ANOVA.

Cell Adhesion Inhibition Assay- This assay was performed using methods described in "Cell Adhesion Assay". Cells were seeded on 24-well culture dishes and incubated for 24h. One group of cells seeded on RGD peptide-grafted substrates contained 0.1mg/ml soluble peptidomimetic in the culture medium. Another group was seeded on peptidomimetic-grafted substrates in the presence of 0.1mg/ml soluble RGD peptide in the culture medium. The other two groups were seeded on RGD peptide or peptidomimetic substrates without soluble peptide or soluble peptidomimetic. The percentage of control adhesion was determined as described in "Cell Adhesion Assay". The average percentage of control adhesion was determined from duplicate independent experiments. Comparisons between samples groups were made using ANOVA.

Enzymatic Stability of RGD peptide and RGD peptidomimetic in solution- GRGDSP and GHDHA were mixed with trypsin solution in HEPES buffer (10 mM, PH=8) to a final solution of 0.5 mM peptides and 0.05 mM (0.12%) of trypsin and incubated at 37 ^{0}C for 1 hour. As control experiment, both peptides were dissolved in the same buffer without trypsin at the same final concentrations and incubated at 37 ^{0}C for 1 hour. Similarly, in another set of experiment the same samples were incubated at room temperature overnight. At the end of incubation period, trypsin activity was quenched by adding inhibitor and samples were analyzed by Fly Zone Mass Spectrometry to assess the extent of degradation.

Results

Cell Adhesion-Promoting Activity of RGD Peptidomimetic GHDHA- Cell culture well surfaces were coated with covalently immobilized GRGDSP peptide or RGD peptidomimetic N-6-(5 guanidino-heptyl)-2, 6 diamino-hexanoic acid GHDHA using surface chemistry described in Method 1. Cell adhesion on each substrate was determined as described in Method 2. Surface immobilization of dextran on tissue culture wells significantly reduced adhesion and spreading of all cell types (Fig. 5; 0.017±0.03 % control). Surface immobilization of GHDHA peptidomimetic promoted extensive cell adhesion and spreading (Fig. 5; 105.9±12.8% control) comparable to substrates containing surface grafted GRGDSP peptide (Fig. 5; 124.3±4.1% control). These results demonstrate the cell adhesion promoting activity of GHDHA peptidomimetic.

Inhibition of Cell Adhesion to Surface Grafted RGD Peptide by RGD Peptidomimetic GHDHA- Soluble ligand competition experiments were performed to demonstrate integrin-binding activity of the GHDHA peptidomimetic. Soluble GHDHA peptidomimetic (0.1mg/ml) completely inhibited cell adhesion and spreading on surface-immobilized GRGDSP peptide

substrates (Fig. 6; 0.08%±0.6% control). This result suggests that soluble GHDHA mimetic bound to integrins competitively to inhibit integrin-mediated adhesion and spreading. Soluble GRGDSP peptide (1mg/ml) completely inhibited cell adhesion and spreading on surface-immobilized GHDHA peptidomimetic substrates (Fig. 6; 5.7%±6.9% control). This result suggests that cell adhesion to surface-immobilized GHDHA peptidomimetic substrates is integrin-mediated and can be inhibited by soluble integrin-binding GRGDSP peptide.

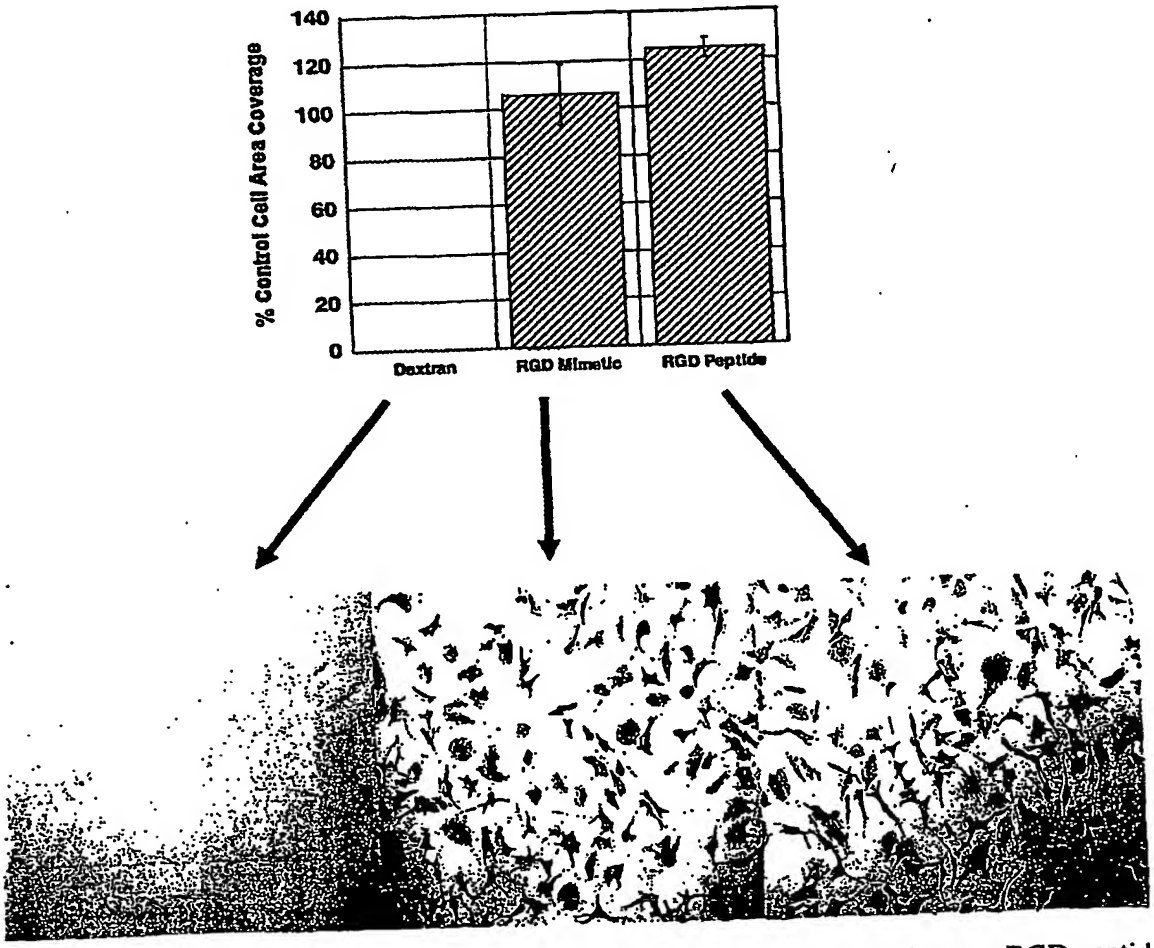


Figure 5 - 3T3 fibroblast adhesion and spreading on surface-immobilized dextran, RGD peptide-grafted dextran, and RGD peptide mimetic-grafted substrates. All data is expressed as a percentage of control adhesion on uncoated tissue culture plastic. Arrows point to representative photomicrographs (100X magnification) of adherent cells on each substrate.

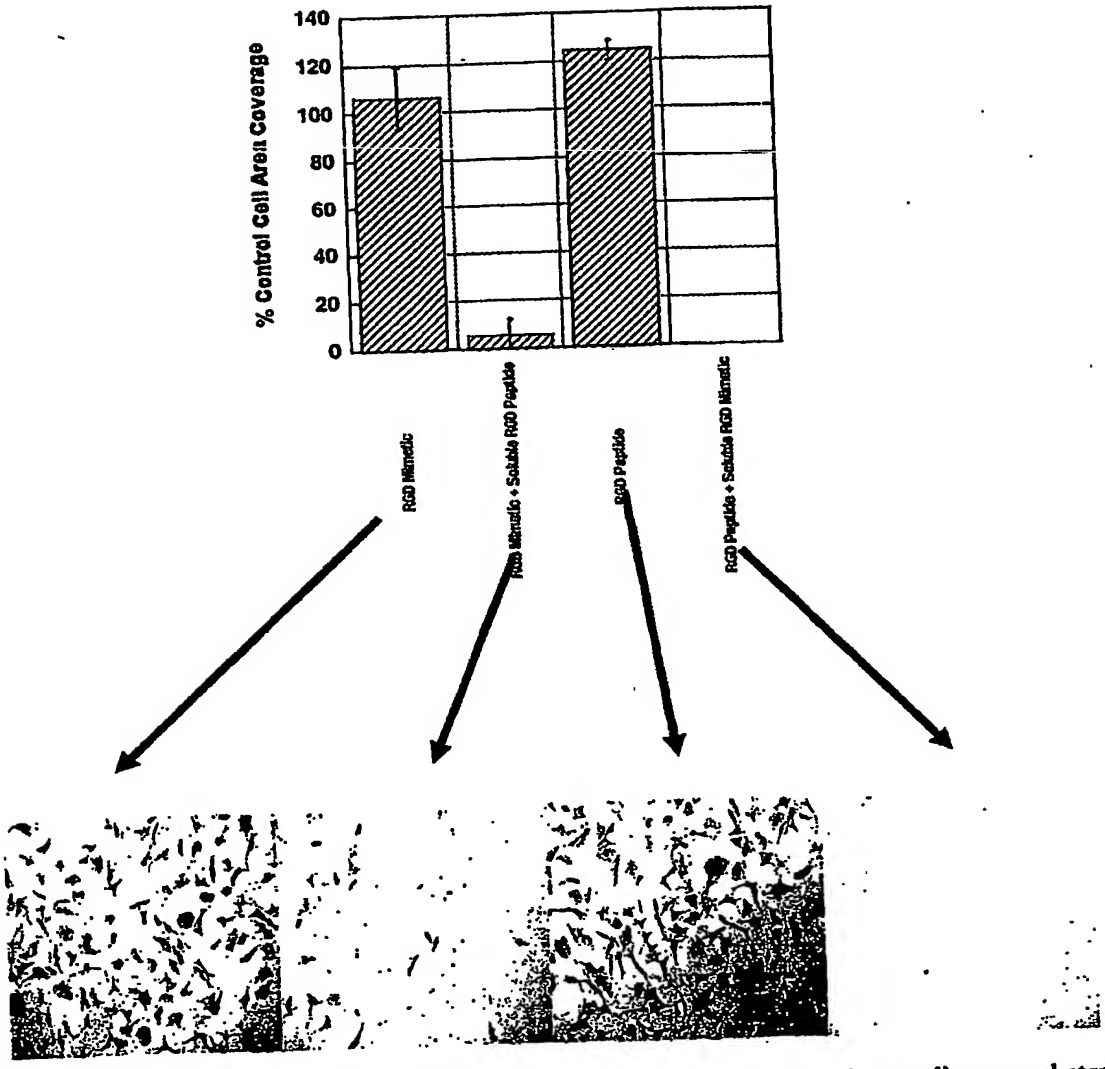


Figure 6 – Effect of soluble RGD peptide on 3T3 fibroblast adhesion and spreading on substrates with surface-immobilized RGD mimetic and effect of soluble RGD mimetic on substrates with surface-immobilized RGD peptide. Arrows point to representative photomicrographs (100X magnification) of adherent cells for each substrate with or without soluble RGD peptide or RGD peptide mimetic.

Enzymatic Stability of RGD and RGD peptidomimetic in solution- GRGDSP and GHDHA were treated with and without trypsin in HEPES buffer solution for one hour at 37°C. At the end of reaction, sample aliquots were analyzed by Fly Zone Mass Spectrometry. Fig.7 shows 588 the mass peak for RGD peptide before and after trypsinization (1h, 37°C). As it can be seen from this figure, there is a sharp decrease (73%) in peak size at mass 588 after treatment with trypsin. In contrast, no significant changes in the mass peak at 303 of the RGD mimetic were observed after trypsinization for 1h at 37°C (Fig.8). The results obtained from enzymatic treatment at 16h, room temperature are shown in Figs. 9 and 10. As it can be seen in Fig. 9, a further decrease in the 588 GRGDSP mass peak was observed indicating 98% degradation of peptide. Fig. 10 mass spectrum shows no significant mass peak change for the RGD mimetic after 16h, room temperature treatment with trypsin, indicating that this molecule is resistant to trypsin-mediated degradation.

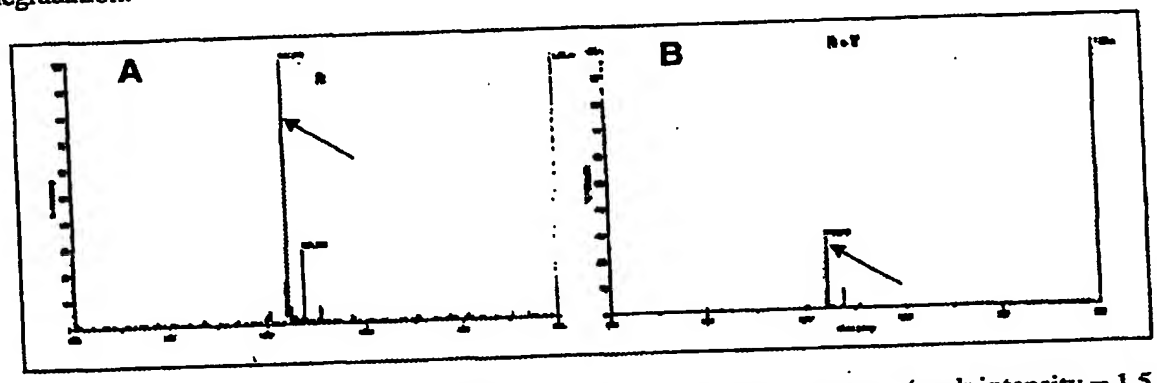


Figure 7. Mass spectrometry of A) RGD peptide prior to trypsin exposure (peak intensity = 1.5 x 10^4) and B) after trypsin exposure (1h, 37°C) (peak intensity = 4.0 x 10^3). The arrows indicate the 588 mass peak of the peptide GRGDSP.

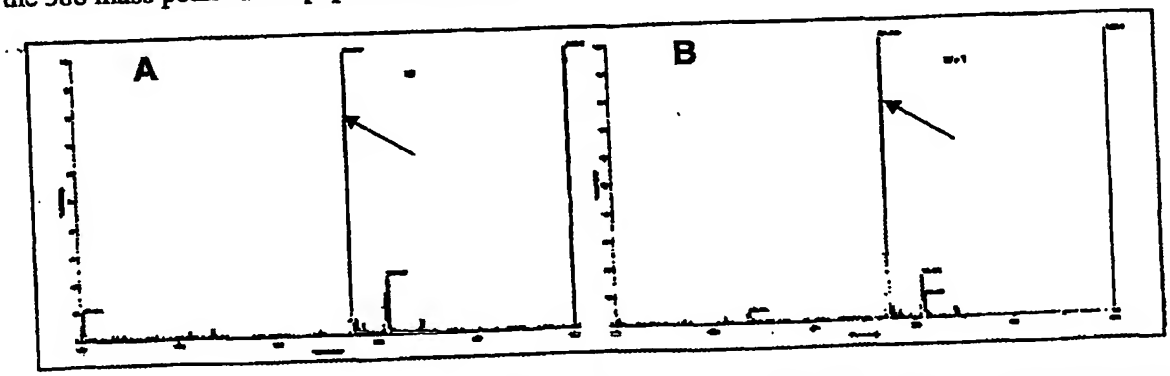


Figure 8. Mass spectrometry of A) RGD peptide mimetic GHDHA prior to trypsin exposure (peak intensity = 1.2 x 10^4) and B) after trypsin exposure (1h, 37°C) (peak intensity = 1.2 x 10^4). The arrows indicate the 303 mass peak of the RGD peptide mimetic.

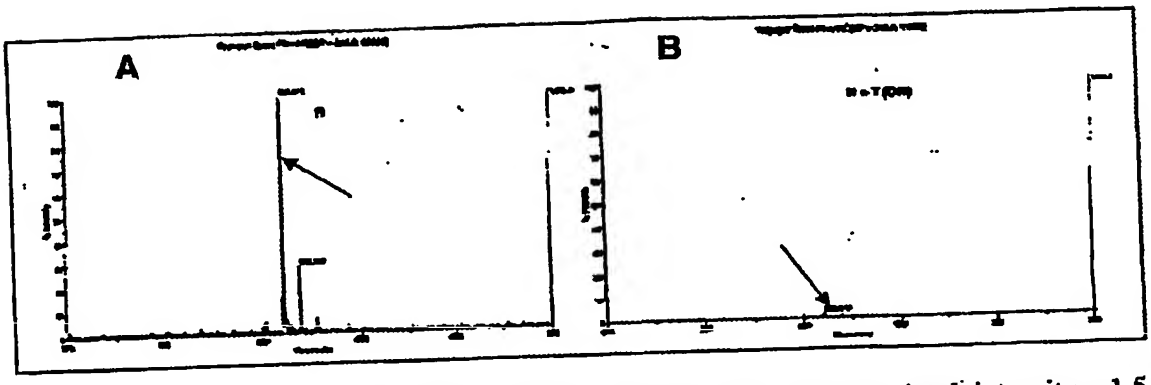


Figure 9. Mass spectrometry of A) RGD peptide prior to trypsin exposure (peak intensity = 1.5 x 10^4) and B) after extensive trypsin exposure (16h, 25°C) (peak intensity = 3.0 x 10^2). The arrows indicate the 588 mass peak of the peptide GRGDSP.

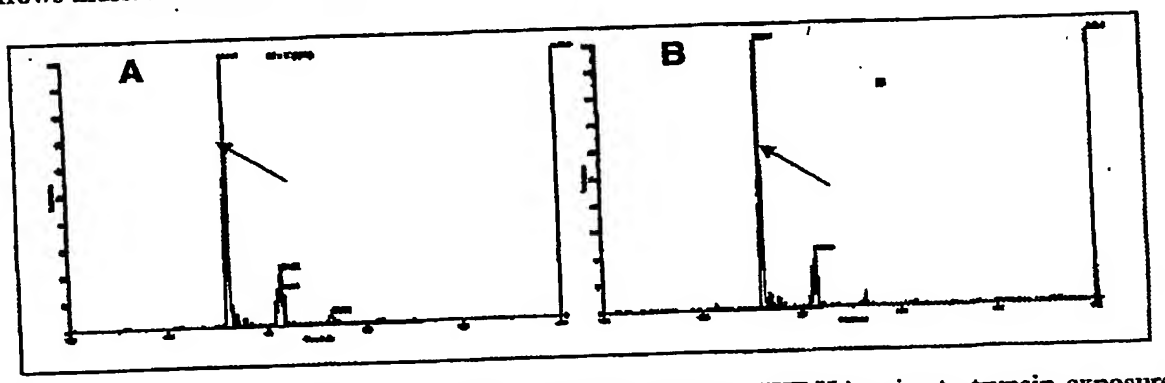


Figure 10. Mass spectrometry of A) RGD peptide mimetic GHDHA prior to trypsin exposure (peak intensity = 1.2 x 10^4) and B) after extensive trypsin exposure (16h, 25°C) (peak intensity = 1.2 x 10^4). The arrows indicate the 303 mass peak of the RGD peptide mimetic.

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We claim:

1. An integrin-binding RGD peptide mimetic compound having the formula N-6-(5 guanidino-heptyl)-2,6 diamino-hexanoic acid.

2. The solid phase process of preparing the RGD peptide mimetic compound of Claim 1 comprising the steps of:

a. supplying a resin comprising immobilized lysine;

b. contacting said resin with the precursor of the compound of Claim 1 wherein said precursor comprises arginine, under conditions whereby the ε amine group on lysine forms an amide bond with the α carboxy group in said arginine;

c. cleaving said amide from said resin; and

d. recovering said compound.

3. The integrin-binding RGD peptide mimetic of Claim 1 immobilized on a solid surface.

4. A method of binding the RGD peptide mimetic of Claim 1 to a solid surface comprising the steps of:

a. coating said surface with dextran;

b. oxidizing said dextran; and

c. contacting said oxidized dextran with said RGD peptide mimetic under conditions whereby said RGD peptide mimetic binds to said dextran coated surface.

5. A method of promoting integrin-mediated cell adhesion to a surface comprising the step of immobilizing the RGD peptide mimetic of Claim 1 on said surface prior to contacting said surface with said cells.

6 A pharmaceutical composition comprising the RGD peptide mimetic of Claim 1.

7. A method of inhibiting integrin-mediated cell adhesion and spreading on a surface comprising the RDG peptide, comprising the step of contacting said integrin with a solution containing the RDG peptide mimetic of Claim 1 prior to contact with said surface.

8. A method of treating a patient for diseases characterized by excessive integrin-mediated cell adhesion comprising the step of contacting said patient with an effective amount of the RGD peptide mimetic of Claim 1 in soluble form.

9. The method of Claim 8 wherein said disease is cancer metastasis, tumor growth, thrombosis, occlusive cardiovascular disease, osteoporosis, retinopathy, renal failure, inflammation, infection or wounds.

10. Biomedical implant material comprising the compound of Claim 1.

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